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Prospective Clinical Evaluation of Amplicor *Mycobacterium tuberculosis* PCR Test as a Screening Method in a Low-Prevalence Population

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Of 656 respiratory samples analyzed for *Mycobacterium tuberculosis* by microscopy, culture, and the Amplicor PCR method, 25 were positive by culture, 12 were positive by microscopy, and 17 were positive by the Amplicor PCR method; 16 samples were Amplicor PCR positive and culture negative. No patient except one with culture-negative, Amplicor PCR-positive samples had clinical indications of tuberculosis. The sensitivity and specificity of the Amplicor PCR compared with those of culture were 68 and 97.4%, respectively. For culture-positive, smear-negative samples, the sensitivity of the Amplicor PCR was 46%.

The majority of routine clinical laboratories still rely on microscopy and culture for the detection of *Mycobacterium tuberculosis* isolates (14). The direct detection of *M. tuberculosis* isolates in clinical samples in a matter of hours is possible through nucleic acid amplification techniques. Studies on the use of PCR for the detection of *M. tuberculosis* organisms show overall good sensitivity and specificity, although the results for sensitivity vary from approximately 50 to 100% (3, 4, 6, 9, 11, 13). Interlaboratory variability of PCR results was confirmed by a recent seven-laboratory blinded study (8). Commercially available PCR kits with specific procedures and controls, like the Amplicor *Mycobacterium tuberculosis* PCR Test (Roche Diagnostics, Basel, Switzerland), may help in reducing interlaboratory variation. We evaluated the Roche Amplicor *Mycobacterium tuberculosis* PCR Test as a first-line screening technique for the detection of *M. tuberculosis* isolates in respiratory specimens in a low-prevalence situation (13.3 cases of tuberculosis [TB] per 100,000 population in 1992).

From May to August 1994, all respiratory samples that contained sufficient material (>1.5 ml) were examined by culture, microscopy, and Amplicor PCR. A total of 656 samples (372 sputum samples, 212 bronchial and tracheal aspirates, and 72 bronchoalveolar lavages) from 102 outpatients and 434 hospitalized patients were examined. An equal volume of 1.45% sodium citrate–0.5% *N*-acetyl-L-cysteine (Janssen Chemica, Beerse, Belgium) was added to each sample, and after being vortexed vigorously, the mixture was left at room temperature for 20 min. Half of the homogenized sample was used for culture and microscopy. About 500 μl of the remaining sample was transferred to an Eppendorf tube, and the tube was stored at −20°C for analysis by the Amplicor method. Samples for culture were decontaminated by the Zephirol-trisodium phosphate method, inoculated onto two Löwenstein-Jensen slants, and incubated for 8 weeks. They were checked for growth once a week. For microscopy, one smear of each respiratory sample was air dried and stained with auramine fluorochrome (Merck, Darmstadt, Germany). The samples analyzed by Amplicor PCR were thawed for half an hour before the start of the assay, decontaminated with sodium hydroxide (final concentration, 1%), and neutralized with 67 mM phosphate buffer (pH 6.8), and the sediment was resuspended in 0.5 ml of phosphate-buffered saline. The PCR was performed according to the manufacturer's instructions. According to the manufacturer's instructions, an A_{450} equal to or greater than 0.35 indicates the presence of *M. tuberculosis* organisms and an A_{450} less than 0.35 indicates the absence of *M. tuberculosis* organisms. We considered samples with an A_{450} equal to or greater than 0.5 to be positive for *M. tuberculosis* and retested samples with A_{450}s from 0.3 to 0.5; samples with A_{450}s less than 0.3 were considered to be negative for *M. tuberculosis.*

Among the 656 specimens tested, culture revealed that four samples contained nontuberculous mycobacteria (one sample contained a *Mycobacterium avium* complex isolate, one contained a *Mycobacterium chelonae* isolate, one contained a *Mycobacterium kansasii* isolate, and one contained an unspecified species). By Amplicor PCR, three of these four samples gave a negative result and one sample that contained *M. avium* isolates gave an A_{450} reading of between 0.3 and 0.5 but became negative on retesting. Only one of these four samples, that infected with the *M. avium* complex strain, was positive on microscopy. Of 652 samples (656 samples minus the 4 samples with nontuberculous mycobacteria), 17 samples (from 9 patients) were Amplicor PCR positive and culture positive and 611 samples (from 504 patients) were negative by both methods. Between culture and Amplicor PCR, there were 24 discrepant results; 16 specimens (from 15 patients) were positive by Amplicor PCR and negative by culture, and 8 specimens (from 4 patients) were negative by Amplicor PCR and positive by culture (Table 1).

All eight samples that were culture positive and Amplicor PCR negative were retested by the Amplicor PCR. Of these eight samples, seven samples (all negative on auramine staining) remained negative on retesting by Amplicor PCR, while one sample, which was also positive on auramine staining, became Amplicor PCR positive on retesting. Examination of patient files (assessment of the patient's history, clinical investigation, chest X ray, and laboratory results) confirmed the diagnosis of TB in all eight patients with culture-positive, Am-

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TABLE 1. Samples with results that were discrepant between culture and Amplicor *Mycobacterium tuberculosis* PCR Test

Sample no.	PCR result	A_{450} value	Culture result[a]	Auramine result[b]	Interpretation[c]	Type of specimen[d]	Age (yr), sex[e]	Relevant clinical data
1	−	0.152	+	−	FN	Sputum	82, M	Pulmonary TB
2	−	0.067	+	−	FN	Sputum	82, F	Miliary TB
3	−	0.069	+	−	FN	BAL	82, F	Miliary TB
4	–[f]	0.081	+	++	FN	Sputum	82, F	Miliary TB
5	−	0.188	+	−	FN	Sputum	82, F	Miliary TB
6	−	0.115	+	−	FN	BA	54, M	Insufficient clinical data
7	−	0.114	+	−	FN	Sputum	64, F	Pulmonary TB
8	−	0.238	+	−	FN	BA	64, F	Pulmonary TB
9	+	0.453	+[g]	+	FP	Sputum	26, F	AIDS, pneumonia
10	+	1.091	−	−	FP	Sputum	26, F	AIDS, pneumonia
11	+	1.149	−	−	FP	BA	49, M	Pneumonia
12	+	1.786	−	−	FP	Sputum	71, M	Pneumonia
13	+	2.277	−	−	FP	Sputum	70, F	Mediastinal tumor
14	+	0.501	−	−	FP	Sputum	81, F	Chronic obstructive pulmonary disease
15	+	1.241	−	−	FP	BAL	3, F	Severe combined immune deficiency syndrome
16	+	0.608	−	−	FP	BA	12, F	Interstitial pneumonia
17	+	1.300	−	−	FP	Sputum	67, M	Chronic obstructive pulmonary disease
18	+	1.877	−	−	FP	Sputum		Chronic obstructive pulmonary disease
19	+	1.487	−	−	FP	Sputum	64, M	Chronic obstructive pulmonary disease
20	+	0.862	−	−	FP	Sputum	70, M	Lung carcinoma
21	+	0.809	−	−	FP	Sputum	64, F	Wegener granulomatosis
22	+	1.877	−	−	FP	Sputum	66, M	Presurgery routine
23	+	0.797	−	−	FP	BA	56, F	Pneumonia
24	+	0.516	−	−	UD	Sputum	58, M	Lung carcinoma, TB in 1956 and 1990

[a] +, weakly positive (less than 10 colonies per slant); −, negative.
[b] ++, more than nine acid-fast rods per 10 fields, but not more than nine acid-fast rods per field (magnification, ×250); +, between one and nine acid-fast rods per 10 fields; −, negative.
[c] FN, false-negative; FP, false-positive; UD, undecided.
[d] BA, bronchial aspirate; BAL, bronchoalveolar lavage.
[e] F, female; M, male.
[f] PCR positive on retesting.
[g] Culture revealed growth of *M. avium.*

plicor PCR-negative specimens. Equally, among the 16 patients from whom specimens were culture negative and Amplicor PCR positive, there were indications of TB in only one patient. This patient (sample 24; Table 1) had proven TB infections in 1956 and 1990. For this patient, TB could not be excluded or confirmed in the period of sample collection.

On the basis of these data, the sensitivity of the Amplicor PCR was 68.0% and the specificity was 97.4% (Table 2). For the test samples (25 of 656 positive samples, or 3.8%), the positive predictive value (PPV) of an Amplicor PCR-positive result was 52% and the predictive value of a negative result (NPV) was 98.7%. However, for the whole of 1994, the proportion of positive samples was 1.52%, reducing the PPV to 28.75%, with the NPV being 99.5%. For the Belgian population (prevalence of 13.3 cases of TB per 100,000 population in 1992) the PPV would be 0.3% and the NPV would be 99.9%.

TABLE 2. Comparison of Amplicor PCR with culture results

Specimen category (no. of specimens)[a]	No. of specimens				Sensitivity (%)	Specificity (%)
	Culture positive		Culture negative			
	PCR positive	PCR negative	PCR positive	PCR negative		
All specimens (652)	17	8	16	611	68	97.4
Smear negative (640)	6	7	16	611	46.1	97.4
Smear positive (12)	11	1[b]	0	0	91.7	100

[a] Four specimens that contained nontuberculous mycobacteria are not included.
[b] PCR positive on retesting.

Of the 652 samples, 12 were smear positive. Cultures of these 12 samples grew *M. tuberculosis* in all cases. By the Amplicor PCR, 11 of the 12 smear-positive samples were positive and 1 was negative. This smear-positive, Amplicor PCR-negative sample became positive on retesting by the Amplicor PCR. Of the 13 smear-negative samples that were positive on culture (sensitivity of auramine staining versus culture, 48%), 6 were positive by the Amplicor PCR and 7 were negative.

The Amplicor *Mycobacterium tuberculosis* PCR Test incorporates several features that make the incorporation of this technique as a routine screening test for *M. tuberculosis* infection in the clinical laboratory feasible. It includes a relatively simple sample preparation step, automated measuring of reaction results in a microwell format, and a means of preventing the carryover of amplicons from previous reactions. The detection step, however, was still quite laborious, complicated, and a possible source of false-positive results because of incomplete washing of the microwells and contamination of the tubing of the automatic washing machine. Any residue of unbound conjugate in the reaction wells that was not washed off could cause a false-positive test result. In practice, a single person could analyze up to 92 decontaminated specimens (one microwell plate contains 96 wells, for 92 clinical samples and four controls) in about 8 h.

In our study we found an overall sensitivity and specificity of the Amplicor PCR (compared with those of culture) of 68.0 and 97.4%, respectively. The PPV of the Amplicor PCR for the patient population from which samples were examined on the basis of a clinical suspicion of TB was 52%. Hence, the Am-

plicor PCR is more sensitive than microscopic examination of auramine-stained smears but is significantly less sensitive than culture on Löwenstein-Jensen medium. Our results are in line with those reported for the Amplicor PCR by D'Amato et al. (5), who found a sensitivity of 66.7% and a specificity of 99.6%. Other investigators (2, 12) have found significantly higher sensitivities of 82.8 and 95.8% and specificities of 100 and 94.8%, respectively.

The false-negative results by the Amplicor PCR might have been due either to the presence of amplification inhibitors in the sample or to a nonuniform distribution of microorganisms in the test sample. Although all samples were homogenized, an uneven distribution of the bacteria in the sample may be caused by the known tendency of *M. tuberculosis* to form cords and clumps. Another possible cause of false-negative results by the Amplicor PCR might be the sampling bias introduced by the fact that only 25 μl of the original sample was used in the PCR procedure, whereas at least the equivalent of 1 ml of sample was used for culture. The 40-fold greater sample volume used in the culture method could explain the greater sensitivity of the culture method and the false-negative results by the Amplicor PCR, especially for paucibacillary smear-negative samples. Use of a greater sample volume in the PCR could reduce the number of false-negative results but could, on the other hand, also lead to more frequent inhibition of the PCR, as shown by An et al. (1). The inclusion of an internal control in every test tube could minimize the possibility of false-negative results by PCR because of inhibitory substances present in clinical specimens. A final factor that might affect the performance of the Amplicor PCR is the freezing of samples for storage and thawing before use. Although freezing-thawing might reduce the viability of bacteria, there are no indications that it has any effect on the structural integrity of bacterial DNA.

Of 12 auramine-positive, culture-positive samples, 11 samples (and, on retesting, 12 of 12 samples) were detected by the Amplicor PCR. Rapid identification of acid-fast bacilli in smear-positive samples is possible with the Amplicor PCR, and given the current low prevalence of multidrug-resistant *M. tuberculosis* in Belgium, it would be of considerable clinical and therapeutic benefit. Automation and a substantially reduced hands-on time seem to be the most important improvements needed to make this a cost-efficient application. However, of 13 auramine-negative, culture-positive samples, only 6 were positive by the Amplicor PCR. D'Amato et al. (5) reported a sensitivity of 55.3% for smear-negative samples. The low sensitivity of PCR for paucibacillary samples has also been reported by Shawar et al. (11) and Nolte et al. (7). The relatively high percentage of smear-negative, culture-positive specimens in our study may therefore partly explain the low overall sensitivity of the Amplicor PCR. Yajko et al. (15) reported that the minimal number of CFU that could be detected reproducibly by the Amplicor PCR was 42, although in some samples even lower numbers could be detected. These 42 CFU in nondecontaminated samples corresponded to 8 CFU recovered in culture after *N*-acetyl-L-cysteine (NALC)–NaOH decontamination, indicating a loss of approximately 80% of the CFU by the NALC-NaOH decontamination procedure. In our study, we used the Zephirol method for decontamination of culture samples. Zephirol selectively destroys many contaminants but has little activity against the tubercle bacilli. Particularly for paucibacillary samples, the Zephirol method is therefore more sensitive than the NALC-NaOH method. The reduced loss of tubercle bacilli with Zephirol decontamination might also explain the low level of sensitivity of the Amplicor PCR (46.1%) for the culture-positive, auramine-negative samples. Contrary to the NALC-NaOH method, the Zephirol method could not be used to decontaminate samples for PCR, because it inhibited the PCR (unpublished data). However, Yajko et al. (15) showed that NALC-NaOH decontamination had no negative effect on the sensitivity of the PCR.

Among the 15 patients from whom 16 Amplicor PCR-positive and culture-negative samples were obtained, only 1 patient had a known history of TB. There was no history of TB in the other patients and the clinical data did not suggest TB, and a 10-month follow-up of these patients did not reveal the development of TB in any of these patients. If we do not take into account the sample from patient 24, the specificity of Amplicor PCR is therefore 97.6%. Our study shows that Amplicor PCR is a fast method for the detection of *M. tuberculosis* isolates in respiratory samples. In particular, the ability to detect *M. tuberculosis* isolates in smear-negative paucibacillary samples is insufficient. We suggest that the sensitivity of the test could be improved by use of a higher sample volume. Use of an internal control could also eliminate false-negative results because of amplification inhibition. We conclude that the Amplicor PCR cannot yet replace culture as a first-line screening method for the detection of *M. tuberculosis* isolates, but it can be used as a rapid confirmation test for smear-positive specimens or in the case of a strongly suspected TB infection (10).

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